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# A Mycosis of the American Lobster, *Homarus americanus*, caused by *Fusarium* sp.[1]

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A mycosis in cultured American lobsters, *Homarus americanus*, is described. The causative agent, a pigment-producing *Fusarium* sp., was isolated from diseased lobsters from an experimental "lobster farm" in New York. Affected lobsters had "black spots" of various sizes on the exoskeleton and appendages and brownish discoloration of the gills. Hyphae and conidia of the *Fusarium* sp. were present in or on these lesions.

## INTRODUCTION

Imperfect fungi belonging to the genus *Fusarium* have been reported from the Kuruma prawn, *Penaeus japonicus*, in Japan (Egusa and Ueda, 1972) and from the pink shrimp, *P. duorarum*, in Texas (Johnson, S. K., unpublished, Fish Disease Diagnostic Lab., Texas A&M Univ., College Station, TX). In *P. japonicus* the condition was called "black gill disease" and was shown to be the cause of serious mortalities among pond-cultured prawn populations. The disease was characterized by the presence of many black spots in the gills. Affected parts of the gills carried septate hyphae of the fungus. Intramuscular inoculation of healthy prawns with conidia of the fungus caused "black gill disease," and fungus was isolated from gill lesions of artificially infected prawns. On many culture media including Sabouraud's medium, the fungus produced a dark brown diffusable pigment (Egusa and Ueda, 1972). Unpublished information by Johnson did not provide sufficient data to compare the *Fusarium* sp. from *P. duorarum* to the one from *P. japonicus*.

A pigment-producing *Fusarium* sp. from the American lobster, *Homarus americanus*, is described that is similar to the *Fusarium* sp. from *P. japonicus*. This species of *Fusarium* is the cause of serious mortalities at an experimental "lobster farm" in Woodside, New York.

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## MATERIALS AND METHODS

### *Source of Infected Lobsters*

On March 19, 1974, a sample of 14 frozen lobsters, *H. americanus*, was submitted by A. Gmiener of Woodside, New York, for disease diagnosis. The lobsters, averaging 86 mm in total length, were either moribund or "fresh dead" when frozen. Originally, the lobsters were obtained as fourth-stage lobsters on July 18, 1972, from the Massachusetts Lobster Hatchery at Martha's Vineyard. At Woodside, New York, the lobsters were reared in a closed water system at a temperature of 18° to 24° C and a specific gravity of 1.023 to 1.026. They were fed an experimental moist diet. The first sign of the disease noted in these lobsters (on April 17, 1973) was "black spots" on the exoskeleton. mortality of lobsters having "black spots" began on June 9, 1973, and has continued to the present (May, 1974). During that period, a 35% loss was attributable to the disease.

### *Isolation of the* Fusarium *sp.*

Isolate cultures were prepared from portions of gill processes of three lobsters. The isolation media used were fluid thio-

glycollate media (Difco)[2] using the method of Ray (1966), and Sabouraud dextrose agar (Difco) enriched with 2% NaCl and 5% homogenized shrimp (Lightner and Fontaine, 1973). Isolation media contained penicillin at 500 units/ml of medium and streptomycin at a concentration of 500 mg/ml of medium to inhibit bacterial growth. Maintenance medium was PYG broth (Difco) with 2% NaCl. All cultures were incubated at 26° C.

*Microtechnique*

Wet tissue mounts of the gills were made of single gill processes and examined microscopically without further preparation.

Tissues for histological study were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned, and then stained with hematoxylin and eosin or periodic acid-Schiff (PAS) using routine histological methods.

## RESULTS

*Early Signs of Infection*

According to A. Gmiener (personal communication), the earliest sign of infection in a lobster was the appearance of white spots on the exoskeleton. These spots usually appeared 6–10 days following a normal molt. Later the spots turned orange and finally black. Lobsters with "black spots" (Fig. 1) exhibited normal behavior and seemed to feed normally. Occasionally, some lobsters autotomized appendages, particularly the chelipeds, after "black spots" had appeared on those appendages. However, lobsters with "black spots" did not survive the next molt. Death invariably occurred just prior to or during molting.

*Morphology of the* Fusarium *sp.*

Twenty-four-hr colonies of the lobster *Fusarium* sp. grown on solid or fluid media were unpigmented or only slightly pigmented. However, by 48 hr a brown diffusable pigment was apparent in both types of media. In 5-day-old cultures, the mycelium had grown completely over the surface of the Sabouraud agar plates and had produced sufficient pigment to stain the medium and portions of the mycelium dark purplish brown. Aerial hyphae and secondary colonies usually remained unpigmented, as did the mycelium in fluid media as pigment diffused from the mycelium as rapidly as it was formed.

Hyphae grown in PYG broth were typically straight, occasionally branched, septate, and were 2.5–5.0 $\mu$m in diameter.

Micro- and macroconidiospores were formed in large numbers in the three media used. Microconidia typically were ovoid or slightly curved, were one- or occasionally two-celled, and ranged from 8 to 15 $\mu$m in length. Macroconidia were canoe-shaped and were typically three- to five-celled, with most being four-celled (Fig. 2). Four-celled macroconidia were 31–49 $\mu$m in length and 3.8–4.5 $\mu$m in width.

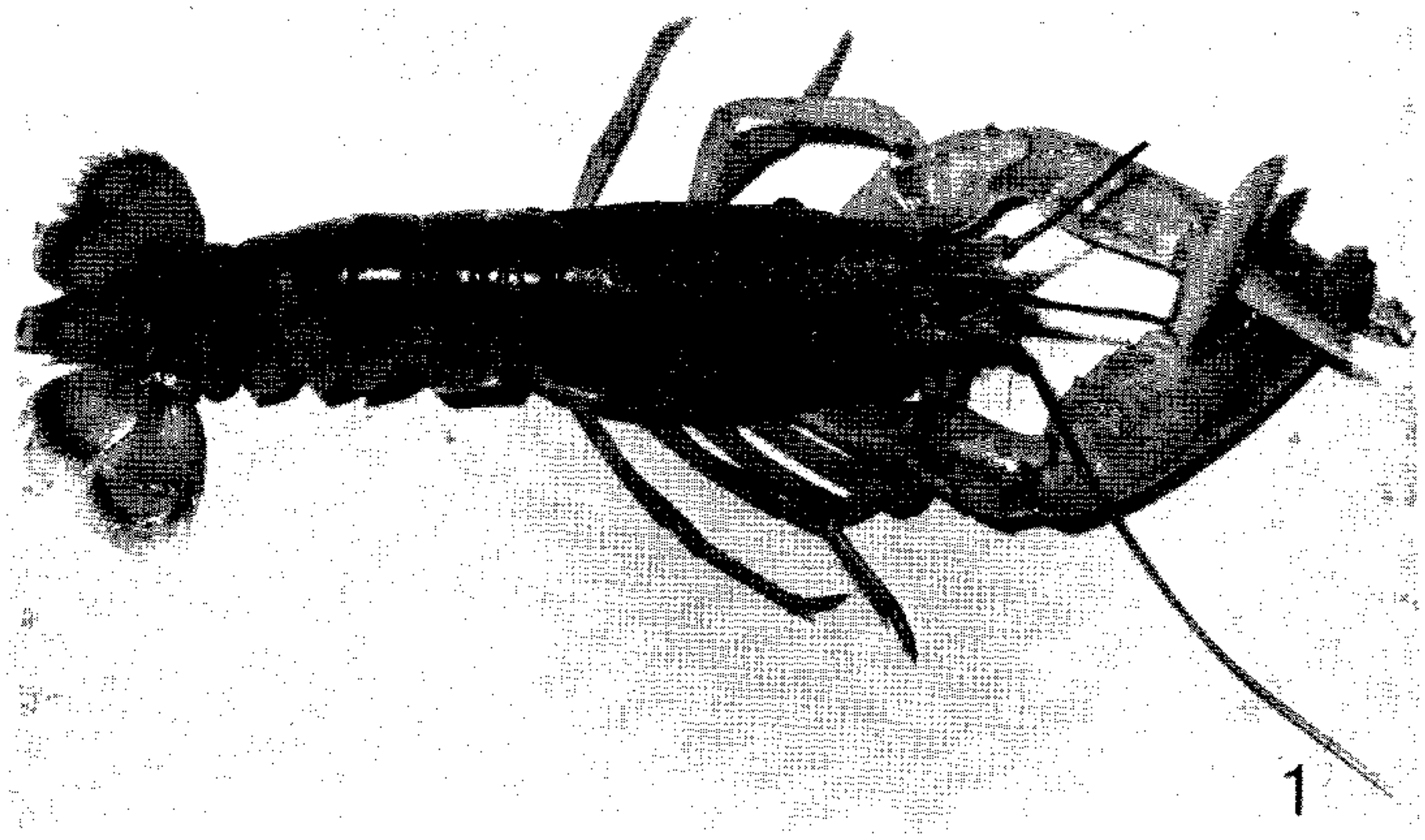
Structures that resembled the chlamydospores described for other species of *Fusarium* (Toussoun and Nelson, 1968) were present in "starved" cultures which had been grown for 7 days in PYG broth and then transferred to sterile water with 2% NaCl and incubated for an additional 24 hr. These structures were round, relatively thick-walled, 6.8–9.4 $\mu$m in diameter, and were formed from the tips and from segments of the hyphae.

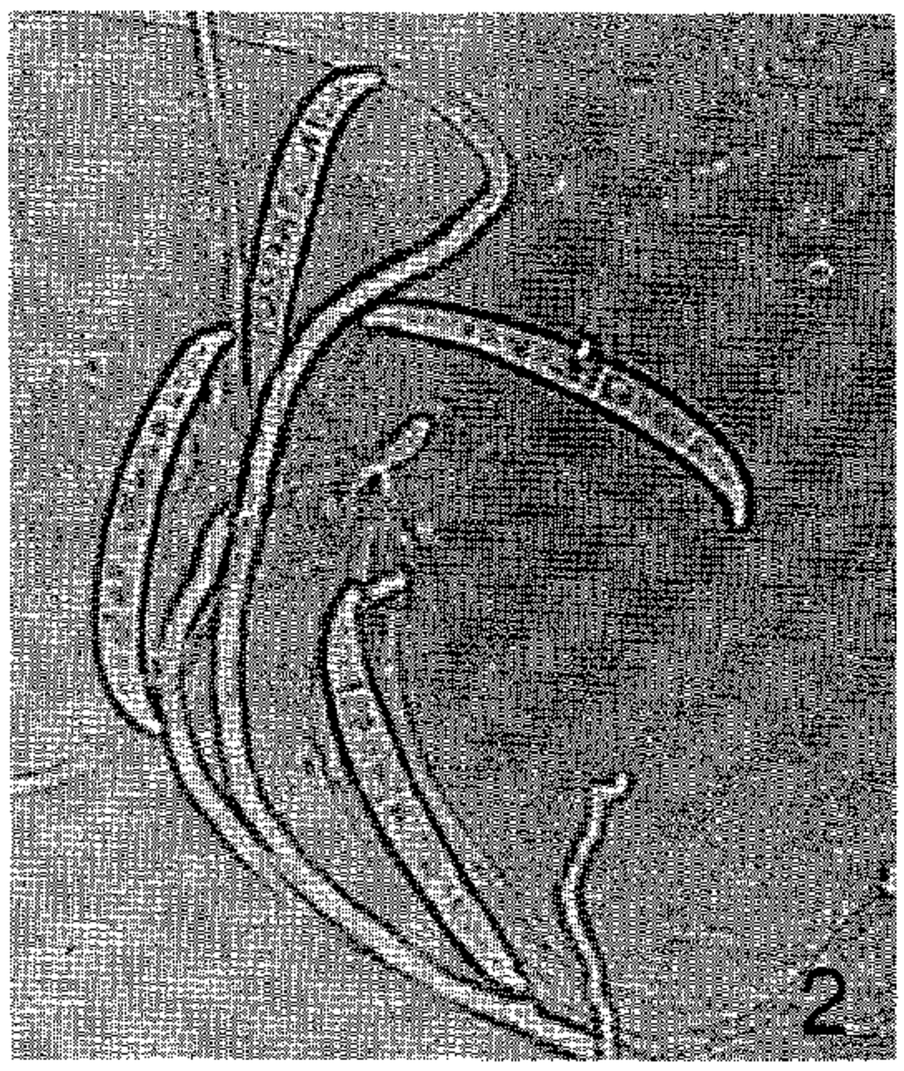
*Pathology*

The gills of all 14 lobsters were discolored. On most of the lobsters the gills were pale brown, but on a few they were a darker brown. None exhibited "black gills" although there were a few scattered "black spots." Wet mounts of gill processes were prepared from ten lobsters and nine of the ten revealed the presence of hyphae and conidia in the gill lamellae (Figs. 3–5). Frequently, hyphae protruded through the tips of the gill lamellae (Fig. 5).

Present in histological sections of the gills and sections of "black spots" on the exo-

[2]The use of trade names in this publication does not imply endorsement of commercial products.





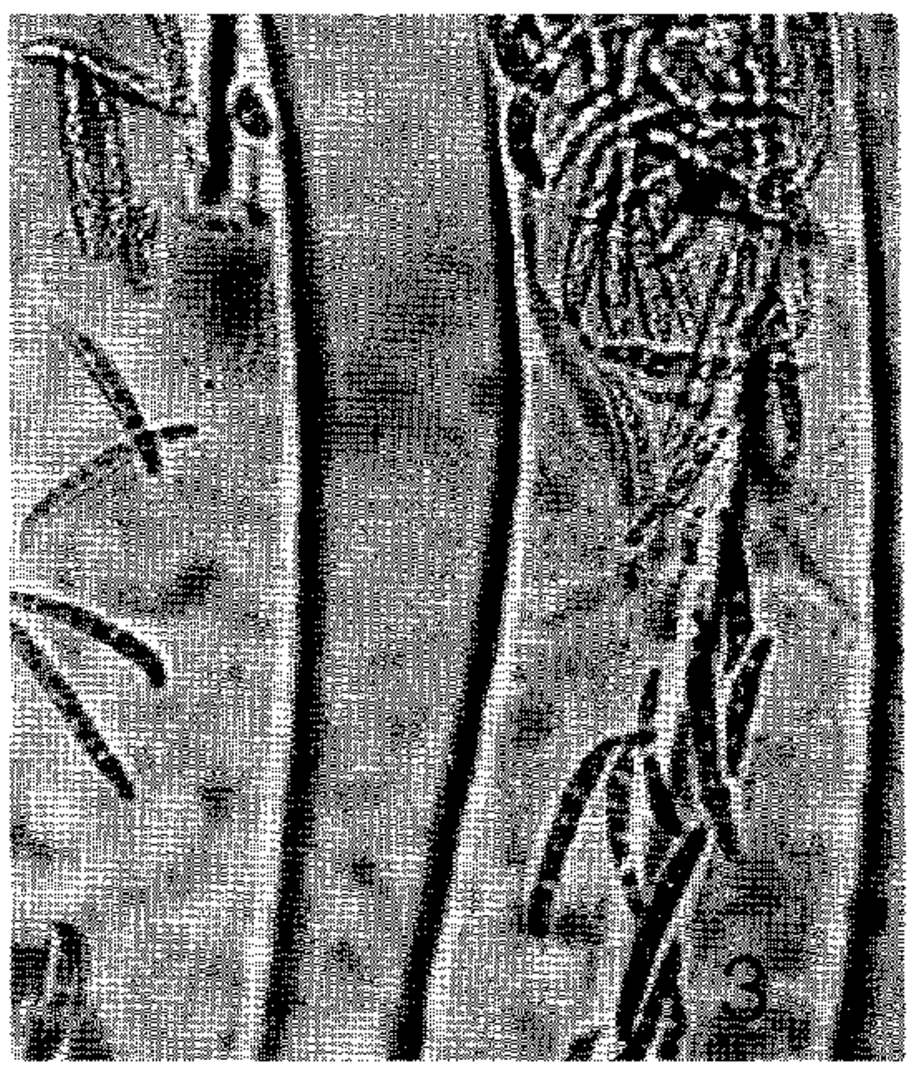


FIG. 1. American lobster *(Homarus americanus)* infected with a *Fusarium* sp. The "black spots" on the exoskeleton are lesions due to the fungus.

FIG. 2. Photomicrograph of four-celled macroconida and hyphae of the lobster *Fusarium* sp. from PYG broth culture. No stain. ×640.

FIG. 3. Photomicrograph of lobster gill lamellae containing only macroconidia of *Fusarium* sp. No stain. ×250.

skeleton were hyphae and occasional macroconidia. The fungus was observed only in the gills and in cuticular and subcuticular tissues of the exoskeleton at the "black spot" lesions. In the cuticular lesions only hyphae were present within the tissues, while macroconidia that were four-, five-, or most often six-celled were produced by aerial hyphae external to the tissues (Fig. 6). In contrast, four- to six-celled macroconidia and some microconidia were formed within the gill lamellae (Figs. 3, 4).

Hemocytic response to the hyphae was pronounced in most of the cuticular lesions (Figs. 6–8), but was less frequently seen in the gill lesions. Hyphae were typically encapsulated when present in the subcuticular tissues by multiple layers of hemocytes (Fig.

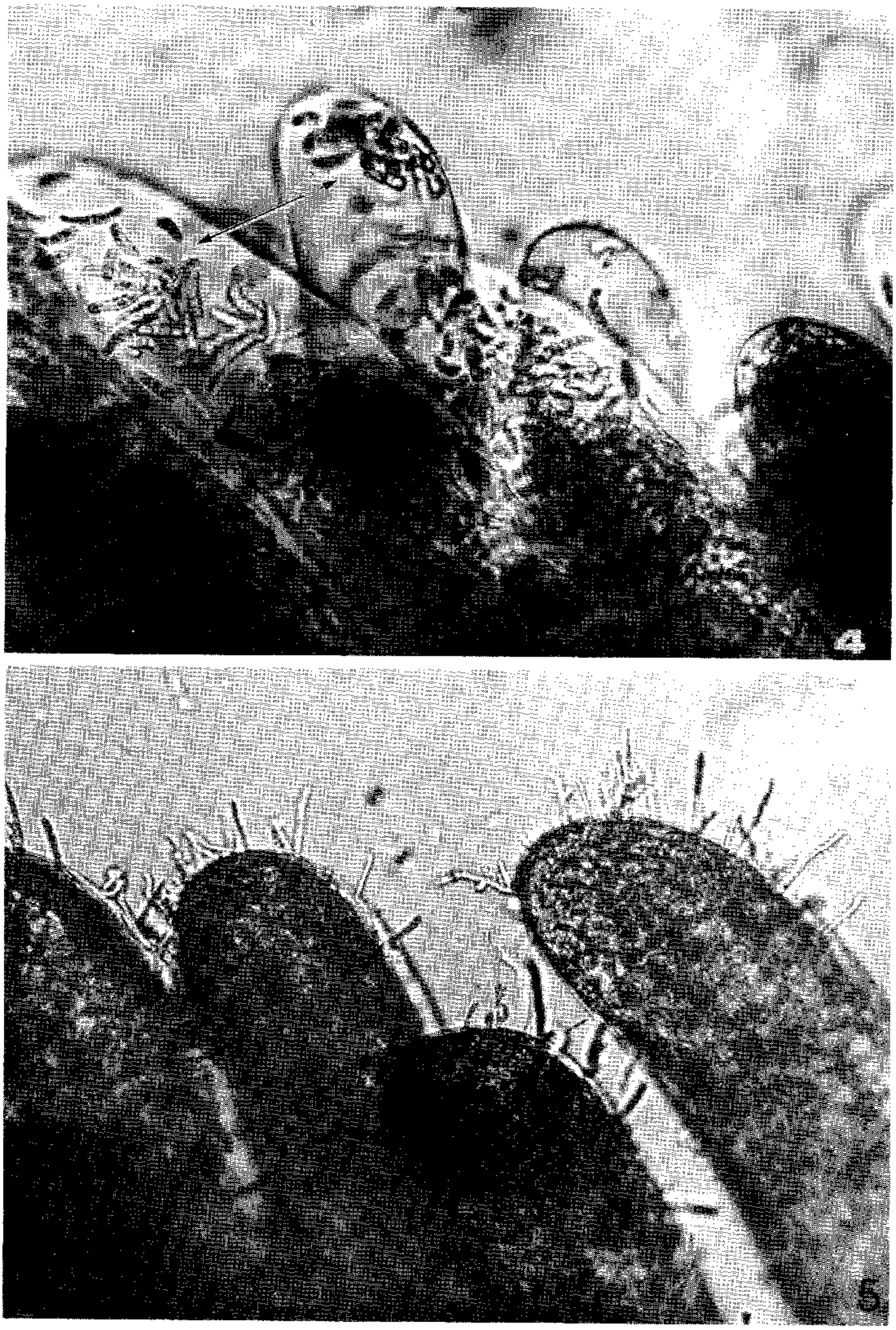
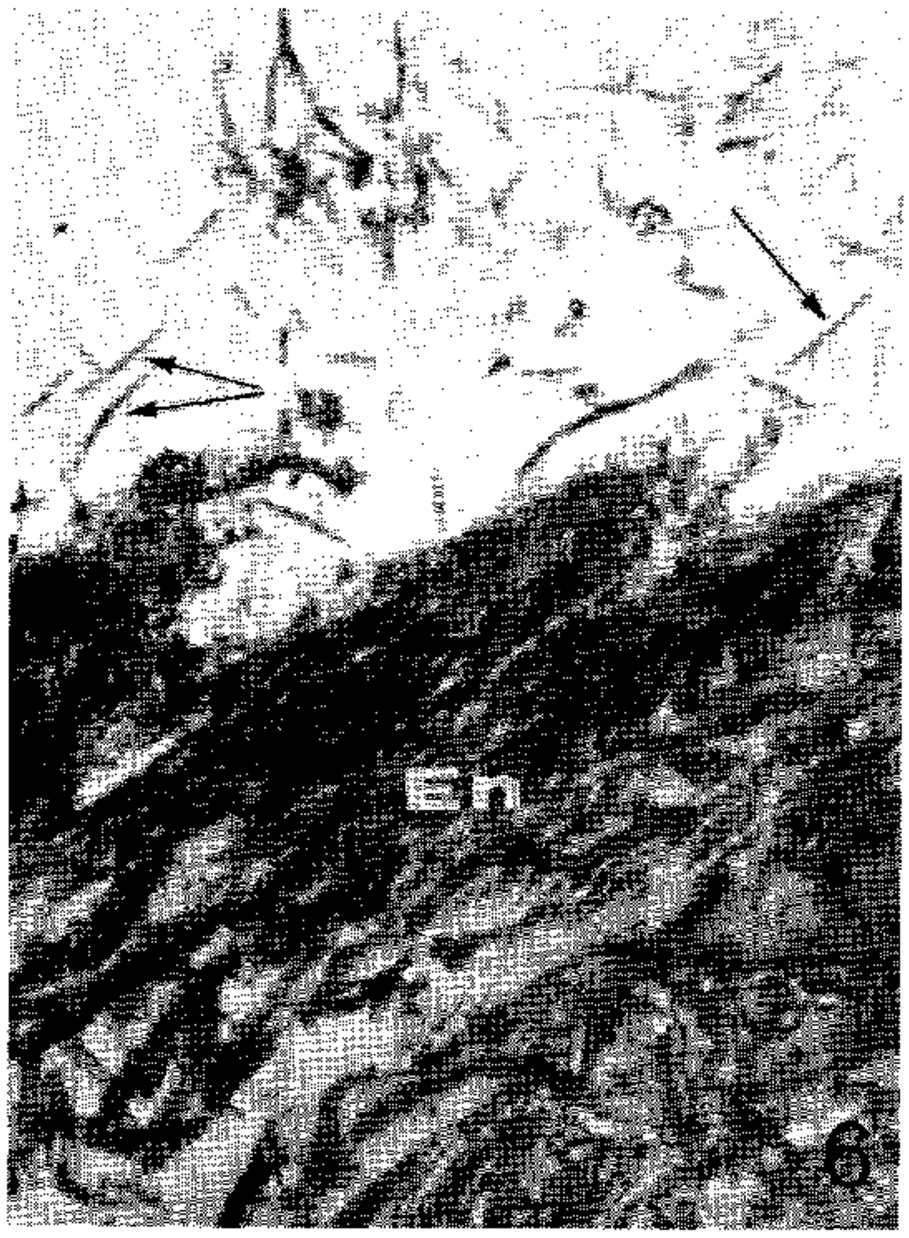
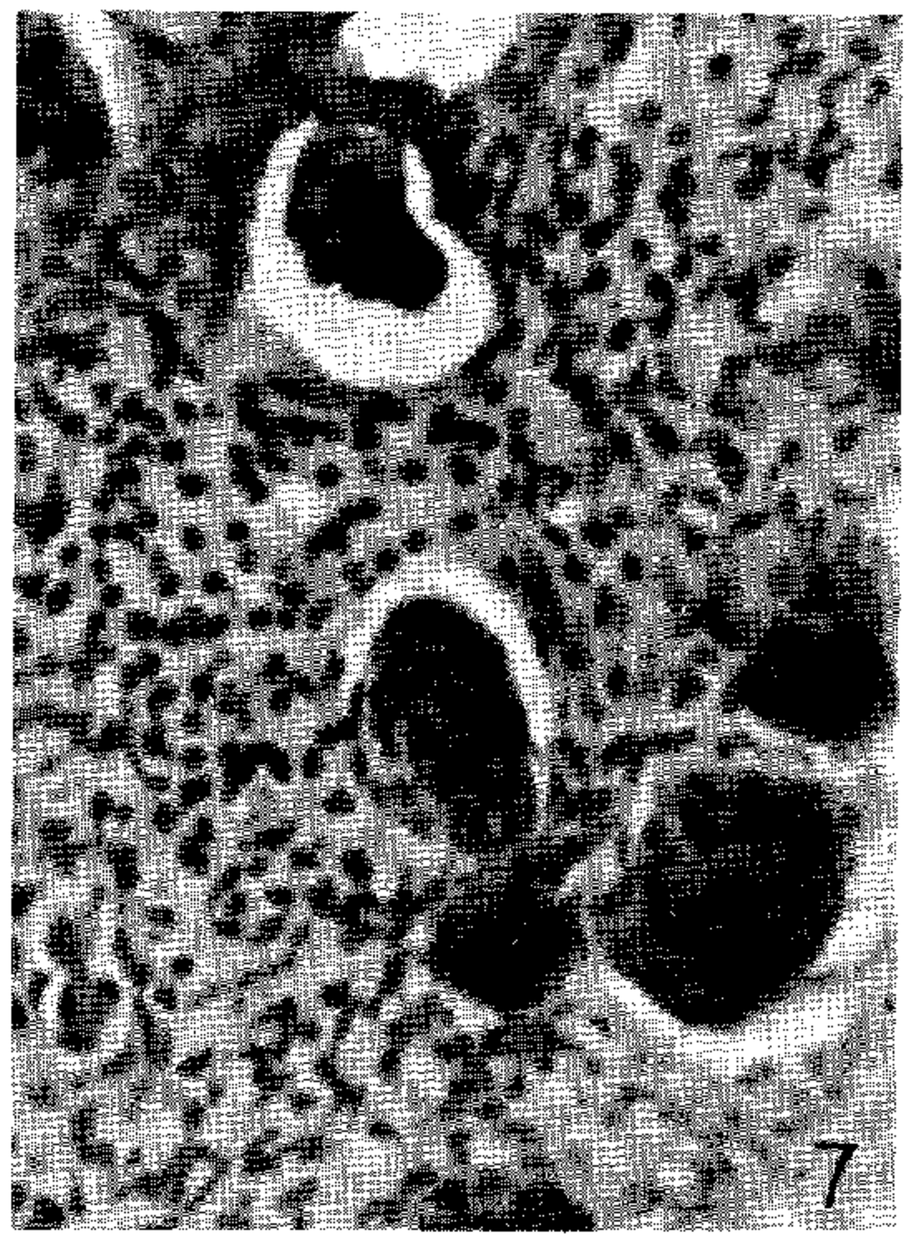


FIG. 4. Photomicrograph of lobster gill lamellae containing microconidia (arrows) and a few macroconida. No stain. ×400.

FIG. 5. Photomicrograph of gill lamellae with hyphae of *Fusarium* sp. protruding through the lamellar cuticle. No stain. ×250.



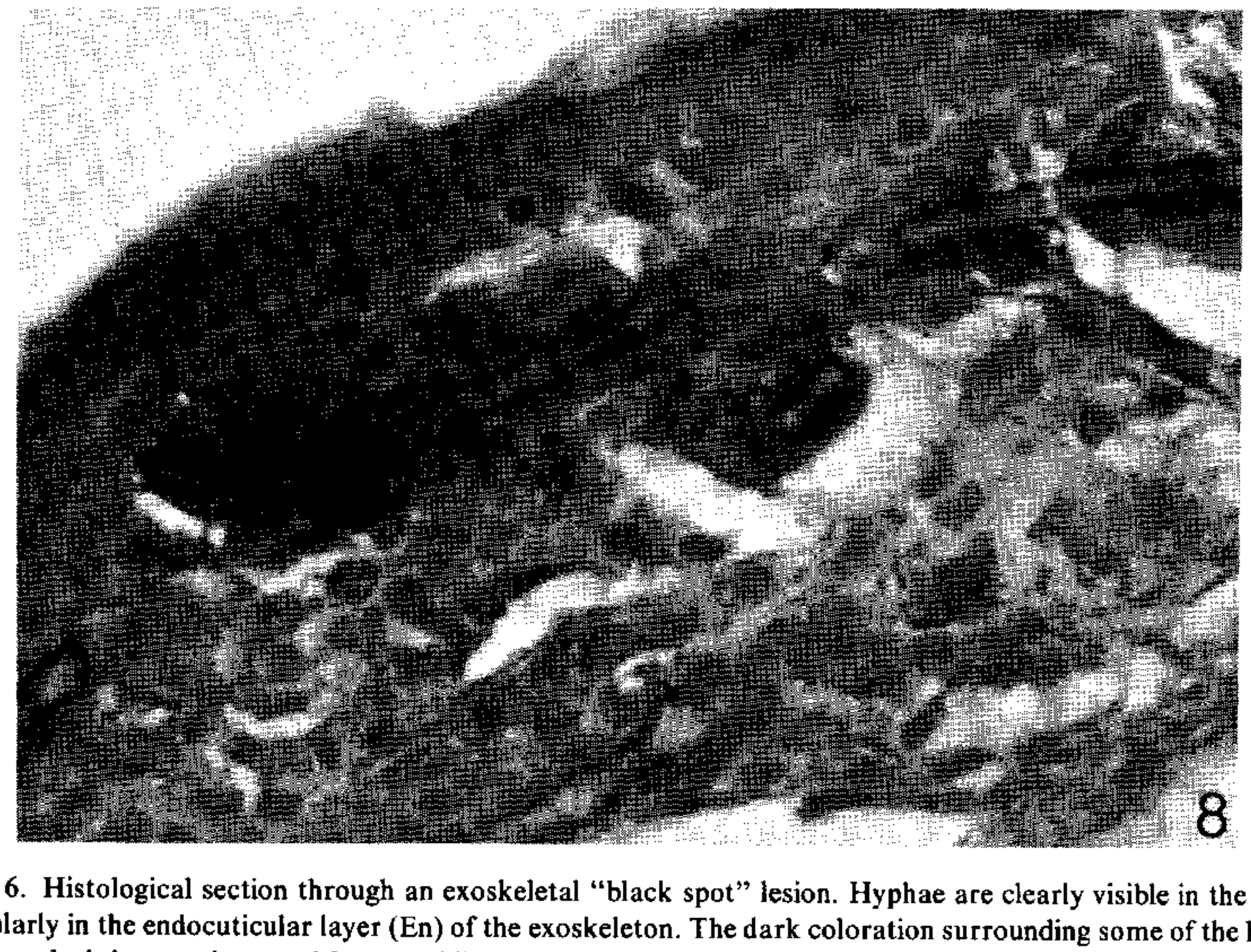




FIG. 6. Histological section through an exoskeletal "black spot" lesion. Hyphae are clearly visible in the lesion, particularly in the endocuticular layer (En) of the exoskeleton. The dark coloration surrounding some of the hyphae is due to a dark-brown pigment. Macroconidia (arrows) are present above the lesion. PAS. ×240.

FIG. 7. Photomicrograph of accumulations of hemocytes around hyphae of the *Fusarium* sp. The dark, pigmented areas around the hyphae are due to a dark-brown pigment. Hematoxylin and eosin. ×380.

FIG. 8. Photomicrograph of hyphae surrounded by a dark-brown pigment and large amounts of amorphous tissue debris that are probably the remains of hemocytes. PAS. ×800.

7). The first several layers of hemocytes forming these encapsulations were commonly melanized. The hemocytes of some crustaceans form melanin in inflammatory processes associated with wound healing (Fontaine and Lightner, 1973) and in response to pathogenic fungi (Unestam and Weiss, 1970; Unestam and Nylund, 1972). Often only melanized debris and hyphae were present in certain portions of the lesions (Fig. 8), but in these areas the debris pigmented by melanin probably represented necrotic and lysed hemocytes.

Hyphae in the exoskeleton, particularly in the endocuticle, were usually surrounded by thin melanized zones although no hemocytes were present near the hyphae (Fig. 6).

The purplish-brown pigment that this fungus produces on Sabouraud medium was believed to have been responsible for the brown discoloration of the gills, but because of the pigment's solubility in water, it does not seem probable that the pigment could remain in the tissues during dehydration and embedding in paraffin. Hence, the pigment observed in histological preparations was melanin formed by the lobster in response to the hyphae and tissue destruction and not the pigment formed by the fungus.

Hyphae and conidia that were not encapsulated, melanized, or associated with hemocytes were frequently observed in the gills (Figs. 3–5). Antemortem growth of the fungus within the gills may have proceeded so rapidly that hemocytic response to the hyphae and tissue destruction was negligible. The destruction of the gills by the fungus was considered to be the cause of death in these lobsters.

## DISCUSSION

Species of *Fusarium* are differentiated mainly on the basis of the shape and size of the macroconidia (Cooke, 1963). Other characters used in differentiating species are the presence or absence and shape of microconidia and the presence or absence of chlamydospores (Toussoun and Nelson, 1968). The *Fusarium* sp. isolated from *P. japonicus* and the lobster *Fusarium* sp. are similar in morphological and cultural characteristics. Both produce macroconidia and microconidia, and a dark-brown diffusable pigment on certain culture media; both cause a form of gill disease in a decapod crustacean. Measurements of the conidia and hyphae are also similar (Table 1). Because of the close similarities of the two isolates, a single species of *Fusarium* may be represented.

TABLE 1
Comparison of Morphological Characteristics of the *Fusarium* sp. from *Homarus americanus* and *Penaeus japonicus*

| | *Homarus americanus* | *Penaeus japonicus*[a] |
|---|---|---|
| Macroconidia: | | |
| Length | 31–49 μm | 30–45 μm |
| Usual number of cells | 4 | 4 |
| Shape | Canoe | Canoe |
| Microconidia: | | |
| Length | 8–15 μm | 6–10 μm |
| Usual number of cells | 1 or 2 | 1 |
| Shape | Ovoid or slightly curved | Ovoid, oblong, slightly curved |
| Chlamydospores: | | |
| Diameter | 6.8–9.4 μm | – |
| Hyphae: | | |
| Diameter | 2.5–5.0 μm | 3.0–4.5 μm |

[a] From Egusa and Ueda (1972).

Fungi belonging to the genus *Fusarium* have not previously been reported from lobsters. However, because mortality can be relatively high in affected populations and because at present no means of treatment, prevention, or control for this disease are known, this disease should be considered to be a potentially serious threat to lobster culture.

## ACKNOWLEDGMENTS

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